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Current Topic

Decreased adherence and spontaneous separation of fetal membrane layers – amnion and choriodecidua – a possible part of the normal weakening process☆

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ABSTRACT

Introduction: The fetal membrane (FM) layers, amnion and choriodecidua, are frequently noted to have varying degrees of separation following delivery. FM layers normally separate prior to rupture during *in vitro* biomechanical testing. We hypothesized that the adherence between amnion and choriodecidua decreases prior to delivery resulting in separation of the FM layers and facilitating FM rupture.
Methods: FM from 232 consecutively delivered patients were examined to determine the extent of spontaneous separation of the FM layers at delivery. Percent separation was determined by the weight of separated FM tissue divided by the total FM weight. Separately, the adherence between intact FM layers was determined. FM adherence was tested following term vaginal delivery (13), term unlabored cesarean section (10), and preterm delivery (6).
Results: Subjects enrolled in the two studies had similar demographic and clinical characteristics. FM separation was present in 92.1% of membranes. Only 4.3% of FM delivered following spontaneous rupture of the fetal membranes (SROM) had no detectable separation. 64.7% of FM had greater than 10% separation. FM from term vaginal deliveries had significantly more separation and were less adherent than FM of term unlabored, elective cesarean section ($39.0 \pm 34.4\%$ vs $22.5 \pm 30.9\%$, $p = .046$ and 0.041 ± 0.018 N/cm vs 0.048 ± 0.019 N/cm, $p < .005$). Preterm FM had less separation and were more adherent than term FM ($9.95 \pm 17.7\%$ vs $37.5 \pm 34.4\%$ and 0.070 ± 0.040 N/cm vs 0.044 ± 0.020 N/cm; both $p < .001$).
Conclusions: Separation of the amnion from choriodecidua at delivery is almost universal. Increased separation is associated with decreased adherence as measured *in vitro*. Increased separation and decreased adherence are seen both with increasing gestation and with labor suggesting both biochemical and mechanical etiologies. The data are consistent with the hypothesis that FM layer separation is part of the FM weakening process during normal parturition.

1. Introduction

Rupture of the fetal membranes (FM) typically follows the onset of uterine contractions; however, in up to 10% of term births, premature rupture of FM (PROM) precedes the onset of contractions. In preterm births, pPROM is the sentinel event in approximately one third of cases and is thus the proximate cause of significant neonatal mortality and morbidity.

The mechanisms by which term or preterm FM weaken and rupture are not completely understood. Previously, we have shown that term FM weaken prior to the onset of labor [1] and that weakening is not solely due to the acute mechanical forces of labor contractions, but rather the result of a biochemical process characterized by apoptosis and extra-cellular matrix remodeling [1,2]. Furthermore, weakening of term FM is not homogenously distributed across the entire surface, but is more localized to the area that overlies the uterine cervix [1,3]. This physiologic "weak zone" is present prior to the onset of labor in the third trimester, as

Abbreviations: FM, Fetal Membranes; ROM, Rupture of Membranes; PROM, Premature Rupture of Membranes; SROM, Spontaneous Rupture of Membranes; AROM, Artificial Rupture of Membranes; SVD, Spontaneous Vaginal Delivery.

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demonstrated from studies of term FM from elective, scheduled, unlabored cesarean sections as well as from spontaneous vaginal delivery (SVD) specimens [1,3]. The acute stretch forces of contractions during the normal labor process may secondarily lead to the rupture of the biochemically weakened FM.

We have observed that layers of the FM (amnion and chorio-decidua) are frequently partially separated at delivery. Significant separation has frequently been a reason for elimination of specific FM from research studies, particularly biomechanical studies, by our group and others. Often this separation is in the region of the weak zone. Additionally, during *in vitro* experiments designed to explore the FM rupture process, we video-documented consistent separation of the amnion from the choriodecidua layers occurring prior to rupture [4]. In the same report we demonstrated that the work (energy) required to rupture FM pieces with fused amnion and choriodecidua is significantly greater than the sum of the work required to rupture adjacent, manually-separated pieces of individual amnion and choriodecidua [4]. Thus part of the work necessary to rupture normal, adherent FM is expended during the separation of FM layers and this separation of the FM reduces the amount of force necessary to rupture the membranes.

Meinert et al. have also reported FM separation and presented specific evidence that changes in the proteoglycans, decorin and biglycan, and especially increases in the glycosaminoglycan, hyaluronan, at the interface of the amnion and choriodecidua contribute to this process [5,6]. They suggested that the changes in decorin and biglycan may result in collagen fiber disorganization and that hyaluronan may absorb water, with resultant increases in tissue pressure, causing FM separation and weakness.

Based upon these observations, we hypothesized that FM separation is a part of the FM weakening process facilitating rupture of membranes. We further hypothesized that if separation of the layers of FM were part of the normal FM weakening process, a mechanism for gradual weakening of the tissue bonds fusing the amnion and choriodecidua, like that proposed by Meinert et al. [6], must exist which would decrease the adherence between the amnion and choriodecidua at the end of gestation. Physical principles dictate that separation of the FM layers should occur when mechanical shear forces exceed the adherence, as determined by these tissue bonds. Thus separation would become more likely as adherence decreases. The aims of this study were to determine the degree of separation of FM layers in normal deliveries and, secondly, to relate the degree of separation to the strength of adherence between the FM layers. To facilitate the latter, we have developed and reported an *in vitro* testing methodology to measure the adherence between intact FM layers [7]. If our hypothesis that FM separation is a normal physiological process is correct, FM from clinically defined groups (i.e., Cesarean section delivery without labor, AROM, SROM, preterm and term) which show greater separation should show parallel decreases in adherence.

2. Methods

2.1. Tissue collection

In vivo study – determination of the normal degree of separation of amnion and choriodecidua: This study was approved by the Institutional Review Board of the MetroHealth Medical Center. MetroHealth Medical Center is a referral center with approximately 3600 annual births, a significant proportion of which are high risk and/or premature births. Following delivery, all placentae with attached FM are routinely stored in a cold storage facility for one week. Placentae are generally placed in cold storage within 30–60 min of delivery. During two study periods of eight weeks duration, during each of the summers of 2006 and 2007, FM from all deliveries were considered for study. Two procedures were followed: 1) For deliveries occurring between 8 am and 5 pm, FM were collected shortly after delivery and examined immediately; 2) For deliveries occurring between 5 pm and 8 am, FM were collected and processed the following morning. FM delivered more than 24 h earlier were excluded. As our objective was to study normal tissue, placentae from labored cesarean sections, with clinical chorioamnionitis, pre-eclampsia, IUGR, FM of multiples, those earmarked for pathological examination for clinical management, and those with gross placental anomalies were excluded. It is not the practice of our pathology service to routinely examine all placentas, thus histological information is not available for FM in the *in vivo* study.

In vitro study – adherence testing: To determine the force of adherence between FM amnion and choriodecidua, we utilized our previously published equipment and protocol to perform standard T-peel testing [7]. FM ($n = 29$) were collected for these studies as follows: term vaginal delivery ($n = 13$: SROM, $n = 7$; AROM, $n = 6$), term unlabored cesarean section ($n = 10$), preterm vaginal delivery ($n = 5$: SROM, $n = 3$; AROM $n = 2$) and indicated preterm unlabored cesarean section ($n = 1$). The same exclusion criteria were used as for the separation study. In addition, FM with histological chorioamnionitis were also excluded. The FM were processed, as described below, within 20 min following delivery.

2.2. Quantification of spontaneous FM separation

FM were cut from the placental disc, washed with Hanks Balanced Salt Solution, removed of blood clots, and then laid flat with amnion side up. Membranes were visually examined for areas of obvious spontaneous separation by teams of two investigators (DK, PS, JS, KB, JN) working together. Separation was usually evident by differences in appearance and translucency between separated versus adherent areas of FM. If an obvious plane of separation was determined and confirmed by both investigators, the amnion was gently manually lifted until the amnion could no longer be lifted without the choriodecidua also being pulled up (Fig. 1A). All areas where the amnion was separated from the choriodecidua were then excised. Hanks solution was gently blotted off the FM prior to weighing. For each placenta, the separated areas (amnion plus choriodecidua) were weighed together. The remaining adherent membranes were then weighed. The proportions of separated FM were reported by dividing the weight of the separated FM by the total weight of the combined (separated plus adherent) membranes.

This methodology assumes that equal surface areas of FM fragments of the same patient have equal weight. FM are known to be heterogeneous, however. Specifically, FM in the area of the weak zone are known to have decreased decidua which may decrease fragment weight [1,8]. The weak zone may also have increased hylauronan with absorbed water which may increase fragment weight [6]. We have compared equal size FM fragments cut from the separated and non-separated regions of the same placenta and found differences in weight/surface area of less than 5% (data not shown).

2.3. Effect of refrigeration storage time upon FM separation

As not all placentae were examined immediately after birth, we determined the possible effects of delay in examination and refrigeration upon spontaneous FM separation. Six FM with varying degrees of spontaneous FM separation were obtained immediately following delivery. Gentian violet was used as a dye to mark along the line of separation between amnion and choriodecicua. The marked FM were then placed in a refrigerator ($-4\,^{\circ}C$). Placentae were re-examined by two investigators every 8 h to assess for